OBSERVATIONS UPON AMPHIBIAN DEUTOPLASM AND ITS RELATION TO EMBRYONIC AND EARLY LARVAL DEVELOPMENT [1]

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During early ontogeny, several distinct morphogenic processes proceed more or less synchronously whereas others tend to alternate (Richards, 1935). In the exponential period, described by Schmalhausen (1930), mitotic activity dominates; but with the onset of gastrulation, the mitotic rate falls in close correlation with an increase in differentiation (initiation of the parabolic period). It is at this time, just as the primary caudo-cephalic axis is about to be laid down, that the first embryonic organizers become evident in the dorsal blastoporal lip (at least in Amphibia) and also that important mitotic centers are set up which feed cells into specific regions where they later differentiate into various anlagen, in some cases, at least, under the influence of induction (Derrick, 1937; Self, 1937; Bragg, 1938; Jones, 1939). Behind these more or less morphological manifestations are the actions of the genes, inductors, possibly hormones, etc. which, working through the visible morphological configurations of the cells or their parts, actually are the basic underlying factors in the production of the embryo, and hence of the adult body.

From these considerations, it is evident that the basic factors in embryonic development are essentially physiological, rather than morphological, in character. Studies of cell-migrations or of morphogenic movements (Vogt, 1929; Wetzel, 1929; Gräper, 1929; Pasteels, 1936; etc.), or mitotic indices (Minot, 1908; Self, 1937; Derrick, 1937; Bragg, 1938; Jones, 1939, etc.), and all similar attacks upon the problem of embryological organization cannot, each method of itself, explain morphogenesis. Such studies are valuable mostly as indicating changes in the morphological configurations of parts which in turn are indirect evidences of the basic physico-chemical changes in the protoplasm, a detailed understanding of which can only be attained by physiological methods. Sometime in the future, therefore, we may expect a synthesis of the observations made by the various methods now in use wherein the relationship between cell division, and

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mitotic centers, induction, cell-migration, increase in size, histological differentiation, problems of cell-size and body-size, nucleo-cytoplasmic ratios, the mode of genic action, etc. will all be correlated into one basic biological principle, only fragmentary glimpses of which any one of us now sees by the results of his own special method.

It is well established that the yolky materials in an egg of an animal constitute reserve food which is utilized during some phase of ontogeny as a source of energy, of building materials, or of both: but at just what phase of development and for what processes they are utilized by the animal has apparently received but slight attention (see, however, Saint-Halaire, 1914). During a recent study of the relation of cell division to early embryonic organization of a toad (Bragg, 1938), it was observed that the yolk granules maintained their initial sizes, shapes, and appearances at least to the stage in which the neural tube closed. From indirect evidence, it was also strongly suspected that the embryo did not increase materially in protoplasmic mass up to this stage of development. If these two conclusions were substantially correct, this could only mean that the yolk was not used during the exponential period nor even during the earlier portion of the parabolic period wherein all of the anlagen of the major organ systems were laid down. In other words, the yolk contributed neither energy for the very actively katabolic process of cleavage nor materials for the increase in the size of the body up to this stage of development in the embryos of the species investigated.

Since these observations were somewhat incidental to the main subject of the former paper, and, further, since the yolk must bear important relationships to some of the ontogenetic processes indicated above, it seemed wise to study the yolk in greater detail in order to establish when and where its utilization begins and, so far as possible by the methods used, for what embryological processes it is utilized. It is also of interest to ascertain whether the species used in the former study (*Bufo cognatus*) is peculiar in these matters or whether other amphibian species manifest the same phenomena.

Materials and Methods

The embryos used were those of *Bufo cognatus* Say, *B. woodhousii woodhousii* (Girard), *Rana sphenocephala* (Cope), and *Scaphiopus hammondii* Baird, all from the vicinity of Norman, Oklahoma.[2] Pre-

[2] I am indebted to the following for the use of slides of embryos and larvae prepared and owned by them: to Mr. Virgil Johnson for all stages of *B. w. woodhousii;* to Dr. Minnie S. Trowbridge for embryos of *Scaphiopus;* and to Mr. Robert Taylor for larvae of *Scaphiopus.*

The species of *Scaphiopus* used is the same as that called tentatively *S. bombifrons* Cope by Trowbridge and Trowbridge (1937). In a forthcoming paper, Dr.

pared slides of early cleavage, of the blastula, and of the gastrula of the California newt, *Triturus torosus*, were studied also for comparison with the anuran embryos.

The methods were those commonly employed for embryological work. Embryos and larvae were fixed in one of several different fixing fluids (Smith's, Goldsmith's, and Bouin's, most commonly), dehydrated with ethyl alcohol, embedded by the method of Hamlett, and serially sectioned (6–12 micra). Heidenhain's haematoxylin, alum haematoxylin, and alum cochineal were the principal stains used. The exact procedure made little difference for the purpose of the study. Observations upon living embryos and larvae of all species used except the newt were also made.

Following the same method as earlier (Bragg, 1938), the yolk granules in selected regions were drawn under oil-immersion lenses by means of a camera lucida, all carefully to the same scale. The pictures so obtained were then compared with each other and with the details of structure as seen in the microscopic fields. The facts

Minnie S. Trowbridge and the author will show that the species name, *bombifrons* is not a synonym for *hammondii* as assumed by Wright and Wright (1933) and that the species in question here is *S. hammondii*.

EXPLANATION OF FIGURES

All figures in the plates drawn by camera lucida and to the same scale in order that they may be compared with one another directly. All are of complexes of yolk granules characteristic of the region given for each except Figs. 44 to 49.

A. Blastula No. 12A1, comparable to Bragg, 1938, Stage *A*.
FIG. 1. Micromere.
FIG. 2. Intermediate zone.
FIG. 3. Macromere.
B. Gastrula No. 263A2, comparable to Stage *C* (Bragg, 1938).
FIG. 4. Dorsal ectoderm.
FIG. 5. Just inside the dorsal lip of the blastopore.
FIG. 6. Anterior ectoderm (opposite the yolk plug).
FIG. 7. Dorsal blastopore region. The blastoporal groove between a cell of the dorsal lip (left) and a cell of the yolk plug.
FIG. 8. Condition a short distance inside the blastopore at the dorsal lip. Condition of yolk intermediate between those shown in Figs. 5 and 10.
FIG. 9. Ventral lip of the blastopore.
FIG. 10. Inner yolk mass.
C. Stage of the crescentic blastopore, No. 54A4, comparable to Stage *B* (Bragg, 1938).
FIG. 11. Micromere near the animal pole.
FIG. 12. Innermost yolk cells.
FIG. 13. Region of the dorsal blastoporal groove; compare with Fig. 7.
D. Neural plate stage. No. 75XA1, comparable to Stage *D* (Bragg, 1938).
FIG. 14. Neural plate.
FIG. 15. Dorsal endoderm.
FIG. 16. Lateral mesoderm.
FIG. 17. Lateral ectoderm.
FIG. 18. Ventral ectoderm.
FIG. 19. Ventral yolk mass.

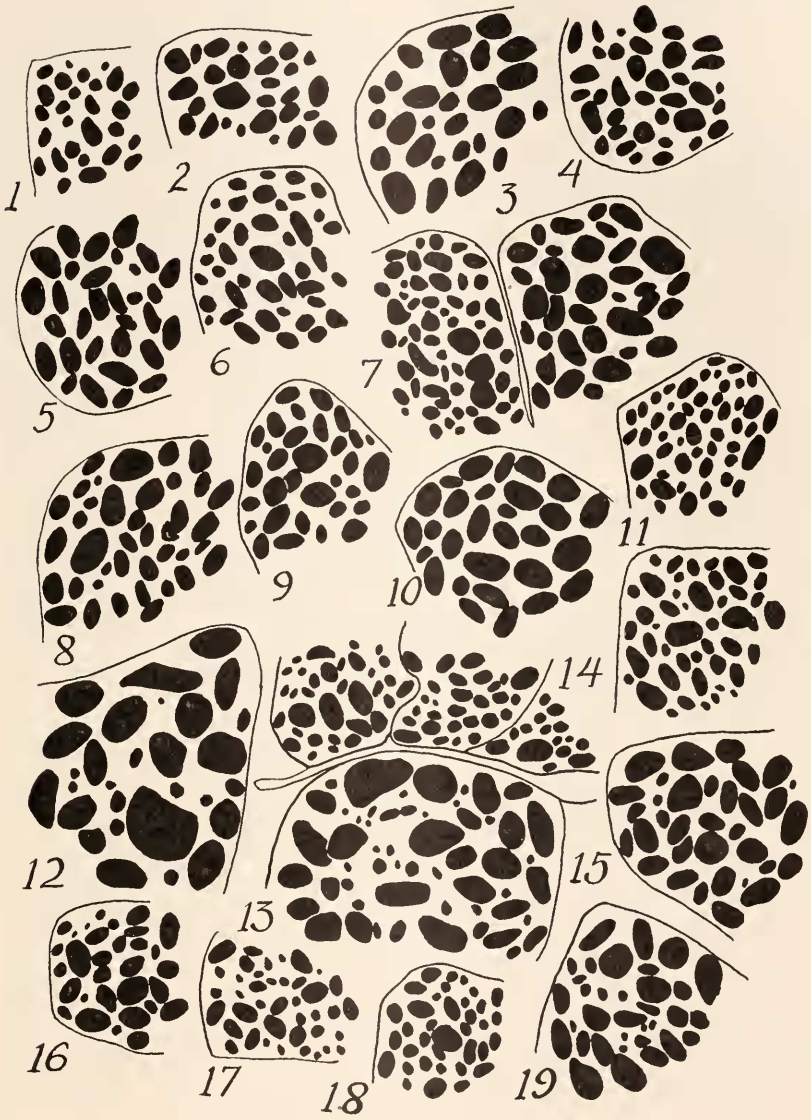

PLATE I. *Bufo cognatus*

gathered in this manner were then correlated with the known stage of development of the individual animals from which the slides had originally been made.

Observations

The distribution and sizes of the yolk granules of all species used followed the general pattern already described for *Bufo cognatus* (Bragg, 1938). Briefly, a gradient of size exists, the smallest granules being mostly located in the animal region, the largest in the vegetal portion of the egg. Species differ in the absolute sizes of the granules but the mode of distribution is the same in all. During cleavage, three types of blastomeres become recognizable, each easily differentiated from the others by the type of yolk granules contained.

Explanation of Figures—Plate II

Fig. 20. Dorsal mesoderm.
Fig. 21. Notochord.
E. Neural tube not quite closed. No. 112A3, comparable to Stage *E* (Bragg, 1938).
Fig. 22. Dorsal mesoderm.
Fig. 23. Superficial lateral ectoderm.
Fig. 24. Neural tube.
Fig. 25. Lateral endoderm.
Fig. 26. Endo-chordo-mesoderm.
Fig. 27. Lateral mesoderm.
Fig. 28. Dorsal endoderm.
Fig. 29. Notochord.
F. Neurula. No. 273a.
Fig. 30. Optical vesicle.
Fig. 31. Ectoderm adjacent to the adhesive organ.
Fig. 32. Brain.
Fig. 33. Adhesive organ.
Fig. 34. Ventral ectoderm.
Fig. 35. Notochord.
Fig. 36. Mesenchyme of the head.
Fig. 37. Lateral mesoderm.
Fig. 38. Ventral yolk mass.
Fig. 39. Lateral ectoderm.
Fig. 40. Dorsal endoderm.
Fig. 41. Somite mesoderm.
Fig. 42. Ectoderm of the head.
Fig. 43. Nerve cord.
G. Larva of 3 mm. total length. No. 134 2-2.
Fig. 44. Ventral yolk mass.
Fig. 45. Mesoderm. Note that the yolk is being used.
Fig. 46. Superficial ectoderm. Yolk nearly gone.
H. Larva of 5 mm. total length. No.141E1.
Fig. 47. Myomere of the tail.
Fig. 48. Section of the nerve cord dorsal to the yolk region (Fig. 44). Yolk granules scattered and small.
Fig. 49. Outline of a fold of superficial ectoderm with only the yolk granules shown.

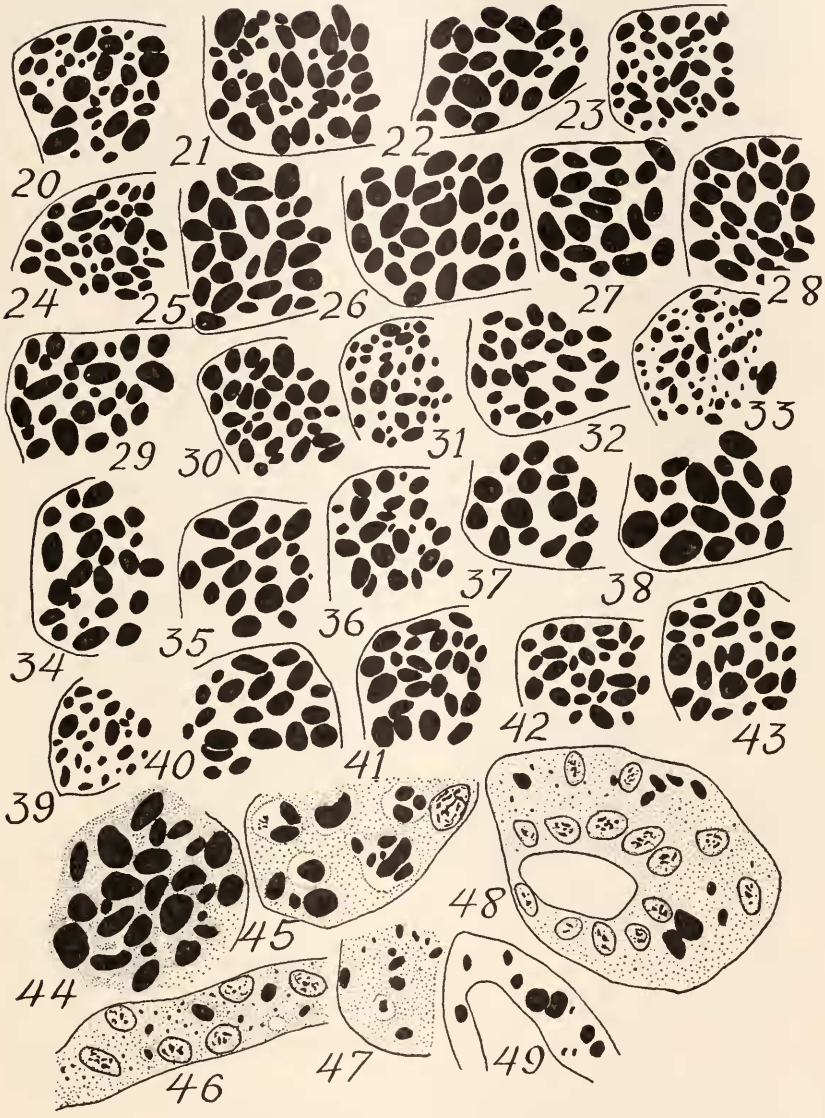

PLATE II. *Bufo cognatus*

These are (1) the micromeres which contain mostly small granules, (2) the macromeres which contain mostly large granules, and (3) cells located in a thick section between these which contain granules mixed and intermediate as to size. This last-mentioned region has been designated as the intermediate zone. These matters are illustrated in Figs. 1–3, 50–52, and 67–68.

I wish to call particular attention to the fact that it is not the absolute sizes of the yolk granules that is to be emphasized. Rather, it is the general appearance of the complex of granules in each type of cell. Some micromeres contain large granules intermingled with the smaller ones and the converse is trûe in the macromeres. The appearance is due in part to a greater number of the one type of granules or the other in any given cell and in part to the average sizes of the granules. The average length of the granules from the regions of the embryo of *Bufo cognatus* shown in Figs. 1, 2, 3, and the two portions of Fig. 7, for example, bear approximately the following relationships to one another: 1.0 : 1.2 : 1.6 : 1.1 : 2.2, the last two figures being for the dorsal lip of the blastopore and the adjacent yolk plug, respectively.

During gastrulation, the yolk granules maintain their original relationships as to size and appearance within each type of cell (Figs. 4–15). A striking contrast between the appearance of the complex of yolk granules in the dorsal lip of the blastopore and that in the cells

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Explanation of Figures—Plate III

A. Blastula No. 1.2a.
- Fig. 50. Micromere.
- Fig. 51. Intermediate zone.
- Fig. 52. Macromere.

B. Neural plate stage.
- Fig. 53. Dorso-lateral endoderm.
- Fig. 54. Ventral yolk mass.
- Fig. 55. Dorso-lateral mesoderm.
- Fig. 56. Ventral ectoderm.
- Fig. 57. Neural plate.
- Fig. 58. Notochord.

C. Open neural groove.
- Fig. 59. Notochord.
- Fig. 60. Lateral ectoderm.
- Fig. 61. Neural fold.
- Fig. 62. Somite mesoderm.
- Fig. 63. Dorso-lateral endoderm.
- Fig. 64. Lateral mesoderm.
- Fig. 65. Ventral yolk mass.

D. Embryo just younger than that from which Figs. 59–65 were taken.
- Fig. 66. Posterior ventral yolk mass.

E. *Triturus torosus*, two-celled stage.
- Fig. 67. Yolk complex near the animal pole.
- Fig. 68. Yolk complex near the vegetal pole.

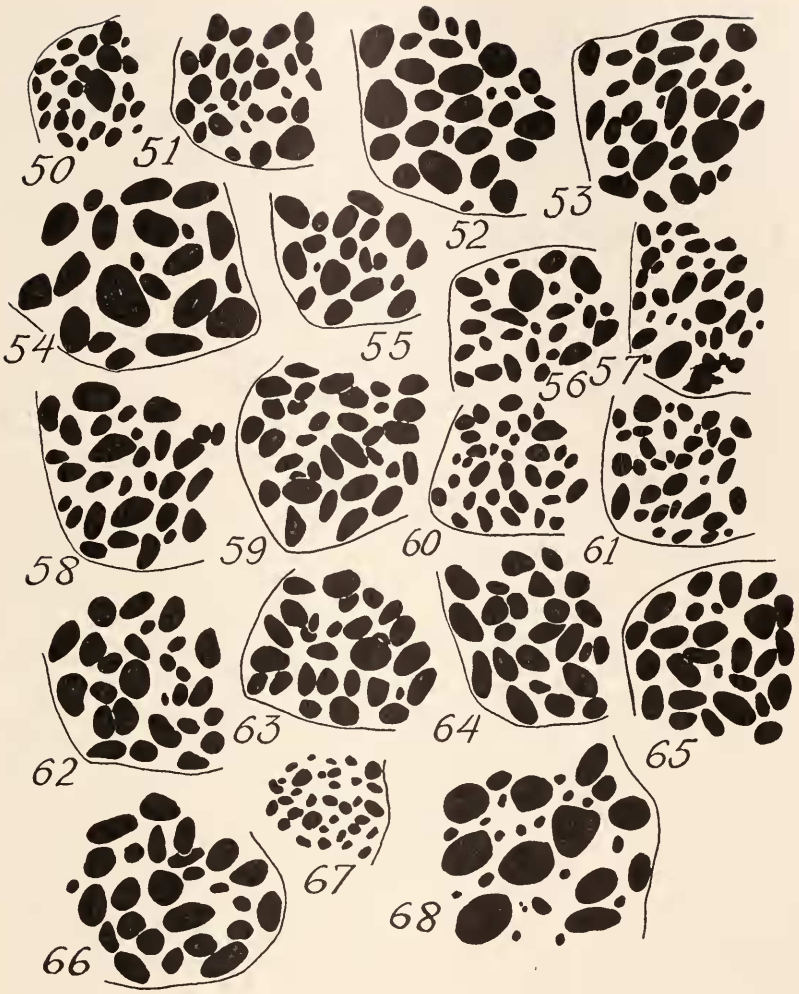


PLATE III. *Bufo woodhousii woodhousii* (except Figs. 67 and 68 which are of *Triturus*).

of the yolk plug just across the blastoporal groove illustrates this fact. (See Figs. 7 and 13.)

In later stages, the essentials in all of the species studied are also as earlier found for *Bufo cognatus*. The micromeres of the blastula pass under the dorsal lip of the blastopore for a short distance only, just enough to cover the yolk plug as the blastopore closes. The micromeres, therefore, give rise mostly only to ectoderm; mesodermal and endodermal derivatives contain only complexes of yolk granules

characteristic of the macromeres of the blastula, except, possibly, some characteristic of the intermediate zone. (See Figs. 14–43 and 59–66.) Hence, it may be concluded without question that in *Bufo*, *Rana*, and *Scaphiopus* and probably also in *Triturus* (stages later than gastrulae not studied) the micromeres differentiate into ectoderm and the remainder of the embryo is derived from the macromeres and the cells of the intermediate zone. Since two orders, four families, and five species appear to agree so closely, it seems very probable that the principles here discussed will be found to apply generally to Amphibia.

In order to determine whether the embryo increases in size during early development, the measurements of embryos and larvae summarized in Table I were made. Taking change in diameter for stages through gastrulation and length thereafter as a measurement of growth, the figures show an increase between the early cleavage stages and the neural plate stage of 33 per cent and a width increase of 16 per cent. At the neural tube stage, the increase in length is 1 per cent more but the width has decreased 16 per cent. Between the neurula and the stage at hatching, the increase in length has reached 136.8 per cent of the diameter at early cleavage and the outer configurations of the embryo have become so irregular that exact measurements of width at any one level of the body-axis can have little meaning. A summary of these facts is presented in graphic form in Text-fig. 1.

It is well known that amphibian embryos absorb water during cleavage. Morgan (1906) found an increase in diameter of about 25 per cent between early cleavage and gastrulation in embryonic frogs, about one-half of which was due to the development of the blastocoel. The figure for *Bufo cognatus* is somewhat less than this (18.9 per cent), probably due to interspecific differences. However this may be, the increase in diameter is not too great to be accounted for almost or quite entirely by the absorption of water during cleavage, particularly if one consider the space occupied by the blastocoel. The data, therefore, confirm the earlier conclusion that no increase in protoplasmic mass occurs up to this stage, although the embryo does actually increase in size. Measurements of living embryos of *Rana sphenocephala* substantiate this general result.

In later stages, but prior to hatching, growth in length is quite rapid but the increase in width is not comparable. Cavities (archenteron, neurocoel, etc.) develop which take up space and the cells become progressively smaller, particularly in areas of high mitotic rate (Bragg, 1938; 1939). While by no means demonstrated, it seems very probable from these considerations that most of the increase in bulk prior to hatching takes place without material increase in funda-

mental protoplasmic constituents (except, of course, the ever present water). This conclusion, moreover, is strengthened by a study of the

TABLE I

Bufo cognatus. Growth of embryos and larvae as measured by length and width after preservation in 70 per cent alcohol. Measurements in mm. to the nearest 0.01. Animals grown in the laboratory at room temperatures (approximately 18–22° C.); cultures maintained in tap water with algae added as food after hatching.

Stage	Age (hrs.)	No. used	Length	Width	Increase between stages		Total increase		Percentage of total increase	
					Length	Width	Length	Width	Length	Width
Early cleavage (2–8 cells)	1–2	20	1.06	1.06	—	—	—	—	—	—
Mid-cleavage	3–6	20	1.12	1.12	0.06	0.06	0.06	0.06	5.6+	5.6+
Gastrulation	18–20	20	1.26	1.26	0.14	0.14	0.20	0.20	18.9−	18.9−
Neural plate stage	33–35	20	1.41	1.24	0.15	−0.02	0.35	0.18	33.0+	16.0+
Neural tube stage	42–46	20	1.42	0.88	0.01	−0.36	0.36	−0.18	34.0−	−16.0+
Hatching	51–55	91	2.51	—	1.09	—	1.45	—	136.8−	—
Mouth a shallow pit	70–74	39	3.00	—	0.49	—	1.94	—	181.5+	—
Mouth a deep pit	74–100	20	3.15	—	0.15	—	2.09	—	197.2−	—
Mouth first functional	102–106	52	4.91	—	1.76	—	3.85	—	363.2+	—
Ready for metamorphosis	45 (days)	22	25.79	—	20.38	—	24.23	—	2285.8+	—

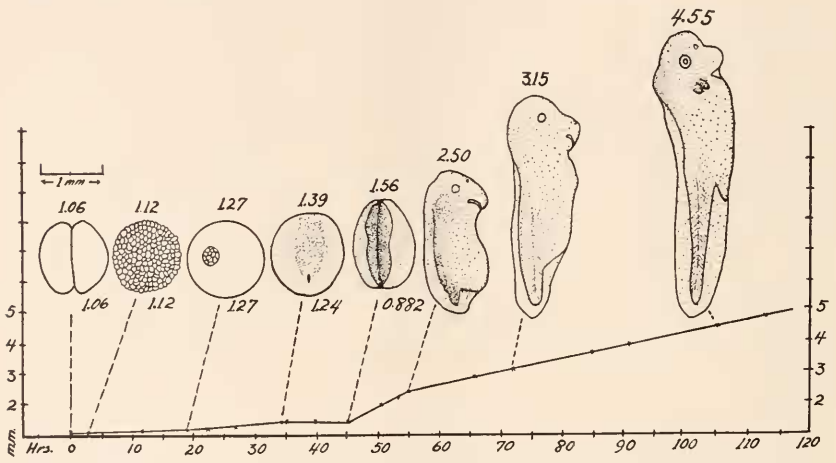
yolk granules in most regions of the embryo, the exceptions occurring during late embryonic life in those areas most active in differentiation.

This is particularly true of *Scaphiopus:* for example, in the adhesive organ of this organism, the yolk granules of a late embryo are noticeably smaller than those in cells from which the anlage of the organ was derived. The same is true of the optic cup. But in the superficial ectoderm of the head and in the brain of the same embryo, they remain essentially unchanged.

From the foregoing observations, therefore, the following general conclusions may be stated:

(1) The embryo increases in size during all phases of development.

(2) This increase does not take place at a constant rate till hatching, after which it does so (at least as measured by length).



TEXT FIG. 1. Graph, increase in average length plotted against age in hours. The figures above the graph are camera lucida drawings, all to the same scale, of representative embryos and larvae of the stages indicated. The numbers above the drawings are the lengths (mm.) of the examples drawn; the numbers below are the widths of these same embryos.

(3) The increase up to the gastrula is due very largely, if not wholly, to the absorption of water, correlated with the space occupied by the development of the blastocoel.

(4) The development of the neurula from the neural plate stage is accomplished with little or no increase in bulk (length increases but width decreases).

(5) The most rapid growth in length occurs between the neurula and the stage at hatching; since the yolk is not altered within most of the cells during the greater part of this period, however, protoplasmic substance is increased but little in the embryo as a whole, even though the bulk of the embryo may increase due to the further absorption of water.

If the yolk is not utilized during early ontogeny, when and for what is it used? Observations upon late embryos and upon early to half-developed larvae of *Bufo cognatus* and *Scaphiopus* indicate the following: (1) The yolk granules begin to break up and to disappear in some regions before they do so in others. (2) Their digestion begins, in general, earliest in those embryonic regions which are first in histological differentiation. (3) Just prior to and during active digestion, vacuoles often appear in the cytoplasm and the yolk granules come to lie in these as though the vacuoles were formed around them: from this it is thought probable that the yolk is digested in intracellular vacuoles into which digestive enzymes pass from the cytoplasm much as in a protozoön.

Figures 30–49 illustrate these processes in *Bufo cognatus*. A cell of the optic vesicle in the neurula contains yolk granules comparable to those in the blastular micromeres (compare Fig. 1 with Fig. 30). In the ventral ectoderm of the same embryo (Fig. 31) they are smaller. In the adhesive organ (Fig. 33) they are not only smaller but also somewhat irregular in shape. The lateral ectoderm contains some irregularly shaped granules but the complexes of yolk in the mesoderm, endoderm, brain, and notochord remain unchanged. (See Figs. 32 and 35–38.) From this it appears that the yolk is used first in ectodermal structures, particularly those in the region of the anlage of the adhesive organ which is soon to differentiate and to function at hatching (Bragg, 1939*a*). In larval stages (Figs. 44–49) the yolk is disappearing in all regions except the ventral yolk mass.

Embryos of *Rana sphenocephala* show similar trends. In late embryos and early larvae, the yolk is beginning to be utilized in the brain, notochord, and the optic vesicle. The superficial ectoderm is probably just beginning to utilize the yolk but there has been no visible change in the mesodermal and endodermal portions. The more cephalic portions of the anlage of the central nervous system begin the use of the yolk before the more posterior portions. This illustrates the use of the yolk in correlation with anterior and cephalic differentiation in general as opposed to posterior and ventral differentiation.

These conceptions are further illustrated by the study of embryos and larvae of *Scaphiopus*. Little if any yolk is utilized before the neural tube is being formed. However, immediately after the neural tube closes, the differentiation of anterior and dorsal structures is well under way. This is especially noticeable in the adhesive organ but it apparently starts in the mesenchyme of the head before it does in the brain or superficial ectoderm in this species. The yolk granules in the

notochord appear slightly decreased in size but those of the posterior and middle ectoderm, somite mesoderm, ventral yolk mass, and endoderm are still unchanged.

Just before hatching, the relation of the disappearance of the yolk to histological differentiation is still more striking. Ectodermal structures and some parts of the mesoderm are losing yolk but endodermal derivatives, for the most part, are not. The dorsal cephalic ectoderm, the optic cup, and the adhesive organ have lost more of the yolk than most of the other parts.

In a 78-hour larva (approximately nine millimeters in total length), differentiation has already reached a functional state in many organs. Some of the potential blood cells have no yolk granules whereas others have a few enclosed in vacuoles. Many contain small particles of yolk with no visible vacuoles around thèm and some have granules which are apparently unchanged. The superficial ectoderm in all parts of the body has lost much of its yolk. In one embryo, two cells were observed in this layer each of which contained a large vacuole in which were located small particles which stained like yolk. The endodermal wall of the gut still largely retains its yolk although a few of the granules are within vacuoles. The adjacent mesothelial wall of the splanchopleure has relatively few granules, some still quite large, others small. The myotomes of the tail are functional at this time. Sections of this region show the yolk to be small in amount and scattered. The cells of the ventral yolk mass contain granules of various sizes, but since some are definitely located in vacuoles, digestion of yolk has probably just begun in this region.

Discussion

In an earlier paper (1938) it was noted that the mitotic centers in the embryos of *Bufo cognatus* often do not correspond to the centers of susceptibility described by Bellamy (1919) in the embryonic frog and by Hyman (1921, 1926, 1927) and Rulon (1935) in other vertebrates. It was also noted that if the interpretation by these authors of the gradients of susceptibility as metabolic gradients be accepted, one seems justified in thinking of the regions of greatest susceptibility as regions where anabolic metabolism dominates katabolic metabolism. If this be granted, then it follows that histological differentiation is also dominated by anabolic as contrasted with katabolic processes, a conclusion in accord with the distribution of mitotic centers and with the *Gesetzmässigkeit* of Schmalhausen. The place where yolk first begins to disappear in the embryo (dorsal and cephalic regions, especially where most active differentiation is occurring) gives further

evidence for this view, for some of these are the very regions which were found by others to be most susceptible to a variety of harmful influences; and they also tend to be the areas of lowest mitotic rate in certain stages (see especially Bragg, 1938, Table V, p. 165).

During cleavage, katabolic processes dominate and the respiratory relationships of the embryo require much oxygen (Bragg, 1939*a*). The source of the energy used during this period is still unknown, but the results of the study presented herein show clearly that the yolk is not used for this function (nor, indeed, for anything else) during this time.

At gastrulation, the metabolism of the embryo becomes differential, dominated in some regions by anabolism, in others by katabolism. This is shown both by the distribution of the mitotic indices at this stage and by the fact that a secondary center of susceptibility is set up in the dorsal lip of the blastopore (Bellamy, 1919). Since, however, the mitotic rate drops very suddenly at this stage, the embryo as a whole is likely dominated by constructive metabolism; but this is probably only another way of saying that the embryo enters the parabolic period of Schmalhausen. It seems probable, also, that early induction is anabolic in character, since the organizer of Spemann is located in the dorsal blastoporal lip and thus coincides with a center of susceptibility. This is, of course, only what one might expect, inasmuch as the fundamental function of induction seems to be the stimulation of cells to construct embryonic parts which would not arise, at least at a given time or place, without it. Furthermore, all of this correlates nicely with the distribution of the mitotic centers in the gastrula (Bragg, 1938, Table III, p. 161).

The time between early gastrulation and the formation of the neural tube seems to be one of great reorganization. The size of the embryo increases only insignificantly (Table I, Text Fig. 1) and the yolk remains inert; but in this short period (about twenty-five hours at ordinary temperatures in *Bufo cognatus* and probably even shorter in *Scaphiopus*), bilaterality is established, the notochordal and mesodermal anlagen make their appearance and the fundament of the whole central nervous system is formed. The distribution of the paths of cell-migrations and other morphogenic movements (Vogt, 1929 and others), as well as the places of greatest mitotic activity, seem best interpreted to mean that this reorganization is brought about almost wholly by cell-migration from specific centers of katabolic (mitotic) activity at specific places and times.

Following closure of the neural folds no further data on the mitotic indices in the amphibian embryo are available at the present time, but

one would expect from the work on other forms that the mitotic rate in the embryo as a whole would progressively decline and that centers of high mitotic index would continue to arise, particularly just prior to the formation of specific anlagen (Derrick, 1937; Self, 1937; Jones, 1939). The rate of growth in length is greatly increased during the period between the neurula and hatching (a period of about ten hours in *Bufo cognatus* at room temperatures), but this involves the disappearance of the yolk from the cells only in the later stages (except in *Scaphiopus*). It seems probable, therefore, that the increase in mass is only slight and the apparent growth is due to the space taken up, in part by the development of cavities within the embryo, in part by decrease in width relative to length, and in part by further absorption of water. The yolk seems to be used at a slightly earlier period by *Scaphiopus* than in any of the other forms studied and this may be correlated with the exceptionally high rate of development which this form has (Trowbridge and Trowbridge, 1937; Trowbridge, 1939). However, even in *Scaphiopus* the yolk is used first by the regions of most active differentiation, mostly dorsal and cephalic in the embryo.

Late in embryonic life, the curve of growth becomes a straight line and from this time on the yolk progressively disappears from the cells, being used last in the large yolk mass ventral to the lumen of the gut.

Whether, in larval stages, the yolk is utilized primarily for histological differentiation or for increase in the bulk of the protoplasm could not be ascertained with certainty, since these two anabolic processes occur together. The methods used in this study could not, therefore, distinguish between them so far as their relations to the disappearance of the yolk is concerned.

Summary

Sections of embryos and of larvae of several Amphibia, representing two orders, four families, and five species indicate that the yolk is carried passively in most cells till late in the embryonic period. Just before hatching in most species, but somewhat earlier in *Scaphiopus hammondii*, disappearance of the yolk begins in the areas of earliest histological differentiation, mostly dorsal and cephalic in the embryo. Since the regions of greatest susceptibility to injury reported by others are often the ones of lowest mitotic rate, it seems probable that histological differentiation is dominated by anabolic, rather than by katabolic, processes, just as growth must be. Similar reasoning shows that embryonic induction in the dorsal lip of the blastopore is also predominantly anabolic. This is indicated by the correlation

of the results of four methods of attack; the *Getzmässigkeit* of Schmalhausen, the mitotic index, studies of cell migration, and differential susceptibility to injurious environments, as reported by various workers, both in Europe and in America.

One interesting result for which no explanation is offered is that the yolk is not used during early ontogeny by any of the forms studied. This leaves no explanation for the source of the energy required by the very actively katabolic process of cleavage. Little if any increase in protoplasmic mass occurs before the yolk begins to be utilized. It is still uncertain whether the yolk serves primarily for increase in protoplasmic mass, for histological differentiation, or for both, since these predominantly anabolic processes proceed concurrently in the late embryonic and larval periods. However, since the process of early embryonic organization and the laying down of most of the fundaments of the major organ systems occur before the yolk is used, it is clear that all of the early morphological manifestations (whatever their individual natures) proceed normally without the aid of the yolk.

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